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Glucose Tolerance, Insulin and Catecholamine Levels in Germfree Rats[1] (39317)

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In the many studies which employ germfree (GF) or gnotobiotic rodents to control the microbial variable, a matter of prime concern is the comparability of their functional and metabolic parameters to those of conventional (CV) animals. GF rats have shown lower cardiac output and metabolic rate than CV rats (1, 2). Thyroid function appears retarded in its development with age, although thyroxine levels in mature GF rats are comparable to those found in their CV counterparts (3). In addition, the microvasculature of GF rats was found to be relatively insensitive to the action of catecholamines and certain bioactive peptides (4, 5). Since definite differences in metabolic rate (1, 2, 6) and metabolic enzyme activity (3, 7) between GF and CV rats have been established, further studies to determine the factors that control homeostasis in the GF rat appear indicated.

Although in their earlier research, Desplaces *et al.* had suspected the GF rat of a "prediabetic state," later studies showed no evidence of impaired glucose tolerance (8). Wiech *et al.* (9), on the other hand, found elevated fasting plasma glucose levels in the GF rat, delayed and decreased insulin secretion, and reduced glucose clearance; these findings were associated with elevated plasma triglyceride levels. To resolve these ambiguities we have determined plasma glucose levels and glucose tolerance in GF and CV Lobund rats of Wistar origin, relating them to plasma insulin levels before and after intravenous glucose load and to the possible implications of the relative catecholamine refractoriness established for the GF animal (4).

Materials and Methods. The fifty and 100-day-old GF and CV male rats of Wistar origin (LOB(Wi)h) used in this study had been reared on sterilized L-485 (grain-soy) diet (10). Germfree animals were maintained in flexible plastic isolators (11), and CV controls were kept in the open colony room.

The animals were fasted for 20 hr preceding glucose tolerance tests. All samples were obtained between 9 and 11 AM. The GF rats were removed from the isolator prior to the experiment. Under prolonged anesthesia (35 mg sodium pentobarbital[2] per kg body weight, given ip), a cannula of PE 50 polyethylene tubing was inserted into the carotid artery. Blood samples collected for glucose determination and for the insulin immunoassay were 0.2 and 0.5 ml, respectively.

Approximately 8 animals per age and status group were used to establish the glucose tolerance curves. In additional 100-day-old GF and CV animal groups of similar size, both glucose and insulin were determined initially. After the initial samples were taken, all animals received 125 mg of glucose per 100 gm of body weight injected through the saphenus vein. Blood samples were obtained at 5, 30, and 60 min following glucose administration for glucose tolerance. In the additional 100-day-old animals, second samples for both glucose and insulin assays were taken only at 30 min. After each collection a small amount of heparin (0.05 ml of 1:10 diluted sodium heparin) was injected into the tubing to prevent coagulation. Between collections the artery was clamped off. Blood glucose was determined by the glucose oxidase method (12).[3]

Immunoreactive insulin was measured by a double-antibody technique of Hales and Randle (13) using an Amersham/Searle[4] ra-

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[2] Nembutal, Abbott Laboratories, Chicago, Illinois.

[3] Glucostat, Worthington Biochemical Corp., Freehold, New Jersey.

[4] Amersham/Searle Corp., Arlington Heights, Illinois.

dioactive insulin kit. Rat insulin obtained from NOVO Industries[5] was used as a standard.

In a separate experiment plasma catecholamine levels were assayed in untreated GF and CV rats by the Anton and Sayre (14) trihydroxyindole method with slight modification (15). To avoid release of catecholamines under the influence of pentobarbital, the rats (100-day-old) were anesthetised briefly by inhalation of Fluothane[6] GF rats were anesthetized within the isolator. Blood was collected immediately by cardiac puncture. The plasma catecholamines were analyzed fluorometrically utilizing a Farrand spectrofluorometer[7] with 5-mm slits in the exciting monochromator and 20-mm slits in the analyzing monochromator. Photomultiplier amplification was maximal.

Results and discussion. There are contradictory reports in the literature regarding the response of GF rats in the glucose tolerance test. Desplaces *et al.* (8) reported that GF and CV rats exhibited a similar tolerance to oral glucose loading, although the initial glucose level of the germfree animal was consistently lower. On the other hand, Wiech *et al.* (9) reported that the fasting plasma glucose level was higher in the GF rat, and remained elevated up to 3 hr after oral glucose administration. Since a number of factors (age, anesthetic, route of glucose administration) can affect the glucose tolerance test (8, 16, 17), we have administered glucose intravenously so that differences in glucose absorption or stress from esophageal intubation would be minimized. Desplaces *et al.* (8) had demonstrated that pentobarbital anesthesia as such has little influence on blood glucose levels of the Wistar rat. This was more recently confirmed by Furner *et al.* for a 25-mg/kg body weight dose level (18). Only at a 50-mg/kg level could a slight but significant increase in blood glucose levels be observed in the Sprague–Dawley rats used in their study. Davidson, however, found no influence even at this level on insulin release after intravenous glucose administration to Sprague–Dawley rats, although 10-min glucose levels were 8% higher than in anaesthetized controls (19). No important effect would be expected from the 35-mg/kg dose used in the present investigation.

The data from 50- and 100-day-old GF and CV rats are summarized in Table I. Since comparison of glucose levels of the 100-day-old GF and CV rats who had additional blood withdrawn for insulin determination with those rats in which only glucose was determined, had shown no influence of the additional sample taking, these data have been combined. The glucose tolerance profiles of GF and CV rats appear quite similar, and comparable to those described for CV rats by DeSantis *et al.* (20). Only the average 5-min value of the 50-day-old CV rats was found slightly but significantly below the averages of the three other 5-min groups. Wiech has reported fasting plasma glucose levels of GF 50-day-old Fischer rats to be higher than CV levels (9). This was not confirmed by the present study.

Plasma insulin levels before and 30 min after glucose administration to 3-month-old GF and CV Lobund Wistar rats are given in Table II. The data for the CV animals fall in the same range as those of Aranda *et al.* (21). Plasma insulin levels in the GF rat tend to be somewhat lower than in the CV animal. However, insulin mobilization in the GF rats appears adequate for the control of plasma glucose levels. The data demonstrate a percentage increase in plasma insulin concentration during the 30 min after load that is comparable to that in CV rats. In both GF and CV rats the percentage increase in insulin concentration matches the increase in blood glucose. Clearance rate and final blood glucose level (1 hr after glucose administration) in GF rats again equal those observed in their CV counterparts (Table I).

It is well documented that catecholamines affect insulin secretion (22). Recently Baez and Gordon (4) have reported that the microvasculature of the GF rat is refractory to catecholamines, and have related this observation to a material (α-pigment) produced mainly in the GF cecum (5). If this phenomenon were to be explained on the basis of a competition for catecholamine receptor sites by circulating α-pigment, it is conceiv-

[5] NOVO Industries, Copenhagen, Denmark.

[6] Ayerst Laboratories, Inc., New York, New York.

[7] Farrand Optical Co., Inc., Mt. Vernon, New York.

TABLE I. BLOOD GLUCOSE LEVELS (mg/100 ml) DURING GLUCOSE TOLERANCE TEST.[a]

Age (days)	Status	Body wt[b] (g)	Time after administration of glucose (min) 0	5	30	60
50			(7)	(7)	(7)	(7)
	Germfree	166 ± 8	123 ± 4	418 ± 15	224 ± 8	185 ± 6
	Conventional	149 ± 20	112 ± 5	359 ± 7[c]	236 ± 6	184 ± 7
100			(17-20)[d]	(8)	(17-20)[d]	(8)
	Germfree	290 ± 28	123 ± 5	433 ± 27	220 ± 8	177 ± 9
	Ratio 30 min/0 min				1.88 ± 0.09[e]	
	Conventional	307 ± 23	122 ± 4	400 ± 15	219 ± 6	183 ± 1
	Ratio 30 min/0 min				1.80 ± 0.06[e]	

[a] Mean ± SE for the number of animals indicated in parentheses.
[b] Mean ± SD for that status and age group.
[c] Significantly different from all other 5-min groups.
[d] Includes animals used for plasma insulin determination.
[e] Average ratio ± SE of individual values.

TABLE II. PLASMA INSULIN CONCENTRATIONS (μU/ml) BEFORE AND 30 MIN AFTER IV GLUCOSE ADMINISTRATION TO 100-DAY-OLD MALE GERMFREE AND CONVENTIONAL RATS.

Time (min)	Germfree	Conventional	P
0	33 ± 4.4[a] (11)	55.0 ± 8.8 (11)	0.08
30	63.8 ± 11 (7)	132 ± 22 (6)	0.03
Ratio 30/0[b]	1.8 ± 0.2 (7)	2.3 ± 0.4 (6)	NS

[a] Mean ± SE for the number of animals indicated in parentheses.
[b] Average ratio ± SE of individual values.

able that plasma catecholamine levels would show a compensatory increase under GF conditions. However, the data in Table III, which compare well with values reported in the literature (23, 24), indicate no significant difference in plasma catecholamine levels between GF and CV rats. It is therefore concluded that in their function as modulators of carbohydrate metabolism, circulating catecholamines would affect GF and CV rats in a comparable way.

TABLE III. PLASMA CATECHOLAMINE LEVELS IN 100-DAY-OLD GERMFREE AND CONVENTIONAL RATS.[a]

Status	Epinephrine (μg/liter)	Norepinephrine (μg/liter)
Germfree	5.76 ± 0.66 (12)	7.13 ± 0.95 (11)
Conventional	6.93 ± 0.74 (10)	6.98 ± 0.79 (11)

[a] Mean ± SE for the number of animals indicated in the parentheses.

Insofar as plasma insulin levels in the GF rat tend to be somewhat below those found in the conventional animal without affecting glucose tolerance, the data appear to agree with views expressed by Desplaces *et al.* (8). Recently Tolbert and Fain have again emphasized the independence of the two gluconeogenic hormones, epinephrine, and glucagon (25). Epand and Douglas (26) have studied the interrelationship between glucagon and insulin, and have shown that when glucagon levels are lowered artificially, normal fasting glucose levels and glucose tolerance will be achieved at lower insulin levels. It could be speculated that the present finding of normal fasting levels and tolerance curves in the presence of somewhat lower insulin concentrations may be caused by a correspondingly lower glucagon output of the GF Lobund Wistar rat. This needs to be investigated. However, the present data indicate that insulin levels in the GF rat are adequate to assure normal plasma glucose and glucose tolerance. There is therefore no reason to believe that insulin insufficiency plays any role in the syndrome of metabolic anomalies demonstrated by the germfree rat.

Summary. Glucose was administered intravenously to 50- and 100-day-old GF and CV rats. Fasting blood glucose levels in GF and CV rats were found to be comparable. Glucose tolerance tests showed that GF and CV rats clear glucose from the blood at a

similar rate. Although insulin concentrations in 100-day-old GF rats tended to be somewhat lower than in CV rats, the percentage increase during the 30-min period after glucose administration was similar, and matched the increase in blood glucose. Levels of plasma catecholamines were analyzed fluorometrically and were found to be comparable in 100-day-old GF and CV rats. It was concluded that insulin insufficiency plays no role in the syndrome of metabolic anomalies demonstrated by the germfree rat.

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